

CERTIFICATION OF A DIESEL PARTICULATE RELATED STANDARD REFERENCE MATERIAL (SRM 1975) FOR PAHs

DIANNE L. POSTER, MARIA J. LOPEZ DE ALDA, MICHELE M.
SCHANTZ, LANE C. SANDER, MARK G. VANGEL^a, and
STEPHEN A. WISE

National Institute of Standards and Technology
Analytical Chemistry Division and ^aStatistical Engineering Division,
Gaithersburg, Maryland 20899, USA

Standard Reference Materials (SRMs) are Certified Reference Materials issued by the National Institute of Standards and Technology (NIST). Three of these materials, SRMs 1975 (Diesel Particulate Extract), 2975 (Diesel Particulate Matter, Industrial Forklift), and 1650 (Diesel Particulate Matter) are diesel particulate-related materials that are well characterized for PAH isomer distributions. SRM 1975 is a methylene chloride extract of industrial forklift diesel particulate matter and it was developed originally in response to the needs of the bioassay community for a natural environmental extract. Thirty-nine PAHs (or combinations of PAHs) were determined in SRM 1975 using various combinations of four different methods of analysis. SRM 1975 will be issued with certified concentrations for eight PAHs. In addition, reference concentrations will be provided for additional PAHs, including many alkyl substituted isomers such as methylphenanthrenes, methylpyrenes, and methylfluoranthenes. Reference values for the mutagenic activity of the extract will also be provided. The approach and results for the certification of PAH concentrations in SRM 1975 are described in this paper.

Keywords: diesel particles; standard reference materials; PAHs; environmental samples; gas chromatography; liquid chromatography

INTRODUCTION

Standard Reference Materials (SRMs) are homogeneous, stable materials that are well-characterized for specific chemicals or physical properties and are very useful for validating complete analytical procedures including extraction, isolation procedure, and quantification^[1, 2]. The National Institute of Standards and Technology (NIST) has developed several diesel particulate-related SRMs. These include SRM 1650, Diesel Particulate Matter; SRM 2975, Diesel Particulate Matter, Industrial Forklift; and SRM 1975, Diesel Particulate Extract. SRM 1650 is a diesel particulate matter SRM that was issued in 1985 with certified concentrations for five PAHs. The material was collected from heat exchangers of a dilution tunnel following 200 engine hours of several four-cycle heavy-duty diesel engines. The material is currently being reanalyzed to provide a greater number of certified analytes. SRM 2975 is a new diesel particulate material, collected from industrial forklifts, that is also currently being certified for PAH concentrations. The results of the recertification of SRM 1650 and the certification of SRM 2975 will be reported elsewhere. In contrast to SRMs 1650 and 2975 which are particulate materials, SRM 1975 is a solution SRM. SRM 1975 is a methylene chloride extract of the industrial forklift diesel particulate matter that was used for SRM 2975. SRM 1975 was developed originally in response to the needs of the bioassay community for a natural environmental extract, i.e., the constituents of interest are naturally present in the extract as well as other solvent extractable organic compounds.

Four analytical methods were used for the determination of PAHs in SRM 1975. Specifically, two gas chromatography/mass spectrometry (GC/MS) methods were used in combination with two liquid chromatography/fluorescence (LC/FL) methods. For the GC/MS methods, the PAHs were separated on: A) a 5 % phenyl-methyl polysiloxane column, and B) a liquid crystalline column. These columns exhibit distinct selectivity for specific PAH isomers. The LC/FL methods consisted of: A) the LC/FL analysis of a discrete PAH isomer fraction (four-to-six ring) that was isolated via normal phase liquid chromatography, and B) the LC/FL analysis of a total PAH fraction. The

approach and results for the certification of PAHs in SRM 1975 are described in this paper. Similar approaches have been used to certify PAHs in a variety of environmental matrix SRMs^[2, 3], including SRM 1941a (Organics in Marine Sediment)^[4] and SRM 1974a (Organics in Mussel Tissue (*Mytilus edulis*))^[5, 6].

EXPERIMENTAL

SRM 1975 was prepared in 1993 by Soxhlet extracting 5.7 kg of diesel particulate material with methylene chloride. The particulate material was collected from a filtering system designed for diesel-powered forklifts. Following extraction, the solution was ampouled with each amber glass ampoule containing approximately 1.2 mL of solution. For the determination of PAHs in SRM 1975, multiple ampoules were selected for analysis based on a stratified random sample of all ampoules (ca. 4600) to reduce the likelihood of bias due to material heterogeneity. Figure 1 illustrates the analytical scheme.

For Method 1 (GC/MS 5 % phenyl-methyl polysiloxane),

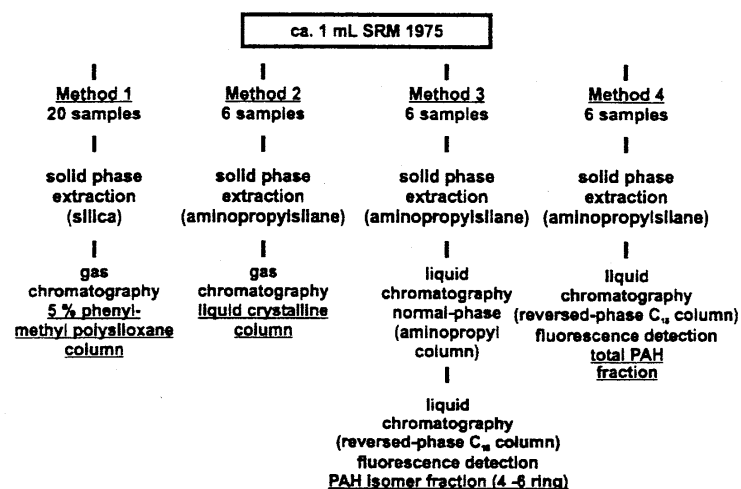


FIGURE 1 Analytical scheme for the determination of PAHs in SRM 1975 (Diesel Particulate Extract).

duplicate subsamples of the extract from each of ten ampoules were passed through silica solid phase extraction (SPE) columns using 2:98 (volume ratio) methylene chloride in hexane to remove potential interfering polar constituents from the diesel extract. Each SPE column was rinsed with the mobile phase prior to its use. Eluents from each SPE column were collected in clean (baked, 500 °C, 18 h, solvent rinsed) glass evaporation vessels and concentrated to 0.5 mL under nitrogen. Samples were exchanged to hexane during the evaporation process and subsequently transferred to amber vials and stored at 4 °C until quantification. The hexane extracts were analyzed by GC/MS using a 0.25 mm x 30 m 5 % phenyl-methyl polysiloxane capillary column.

For Method 2 (GC/MS liquid crystalline), subsamples from each of six ampoules of SRM 1975 were passed through aminopropylsilane SPE columns as described above for Method 1. However, the eluents were evaporated to 1 mL and the SPE procedure was repeated. The eluents were analyzed by GC/MS using a 0.2 mm x 25 m liquid crystalline column coupled with a 2.5 m retention gap. This chromatographic column offers unique selectivity on the basis of molecular shape for the separation of PAH isomers relative to the 5 % phenyl-methyl polysiloxane column, although it has a limited temperature range. Therefore, only a select range of analytes can be readily determined with this column.

Two sets of LC/FL results, designated as Method 3 (LC/FL PAH isomer fraction) and Method 4 (LC/FL total PAH fraction) were used for the certification of PAHs in SRM 1975. For Method 3 (LC/FL PAH isomer fraction), subsamples from each of six ampoules of SRM 1975 were passed through aminopropylsilane SPE columns using 2:98 (volume ratio) methylene chloride in hexane, concentrated under nitrogen, and fractionated on a semi-preparative aminopropylsilane column to isolate a discrete PAH ring fraction (based on the number of aromatic carbons) according to retention times obtained using PAH standards. A mobile phase of 5:95 (volume ratio) methylene chloride in hexane was used for the semi-preparative LC fractionation. A fraction containing four-to-six ring PAHs was isolated for each SRM 1975 sample, concentrated, and analyzed by reversed-phase LC/FL using a polymeric octadecylsilane column. This approach has been described previously^[4, 7-9] and is

reviewed by Poster et al.^[3] For Method 4 (LC/FL PAH total fraction), six subsamples from each of six ampoules of SRM 1975 were passed through aminopropylsilane SPE columns, concentrated, and analyzed by reversed-phase LC/FL as described above.

For both the GC/MS and LC/FL measurements, selected perdeuterated PAHs were added to the SRM 1975 subsamples prior to sample preparation for use as internal standards for quantification purposes. For GC/MS analyses the following internal standards were used: naphthalene-*d*₈, biphenyl-*d*₁₀, phenanthrene-*d*₁₀, pyrene-*d*₁₀, benz[*a*]anthracene-*d*₁₂, and benzo[*a*]pyrene-*d*₁₂. For the LC/FL analyses the following internal standards were used: triphenylene-*d*₁₂, benz[*a*]anthracene-*d*₁₂, perylene-*d*₁₂, and benzo[*ghi*]perylene-*d*₁₂ (LC/FL PAH isomer fraction) and phenanthrene-*d*₁₀, fluoranthene-*d*₁₀, benz[*a*]anthracene-*d*₁₂, and dibenz[*a,h*]anthracene-*d*₁₄ (LC/FL total PAH fraction). Calibration standards were used to determine response factors of the analytes relative to the internal standards. Calibration standards consisted of aliquots of gravimetrically prepared mixtures of SRM 2260 (Aromatic Hydrocarbons in Toluene (Nominal Concentration 60 µg/mL)) for the GC/MS analyses or SRM 1647c (Priority Pollutant PAHs in Acetonitrile) for the LC/FL analyses; gravimetrically prepared solution of additional compounds not contained in SRMs 2260 or 1647c; and SRMs 2269 and 2270 (Perdeuterated PAH-I and PAH-II, respectively).

RESULTS AND DISCUSSION

Capillary GC columns provide excellent PAHs separations with low background interference which facilitates accurate quantitation of individual PAHs^[3]. The 5 % phenyl-methyl polysiloxane stationary phase is widely used for the determination of PAHs in environmental samples. Solute retention results primarily from dispersion interactions between the solute and the stationary phase, and the resulting separations are often based on boiling point differences. However, when boiling point differences are subtle, some PAH separations are hindered, for example benzo[*b*]- and benzo[*j*]fluoranthene (Figure 2). Liquid crystalline columns offer unique selectivity that is useful for the separation of PAH isomers^[10, 11]. The rod-like liquid crystalline groups

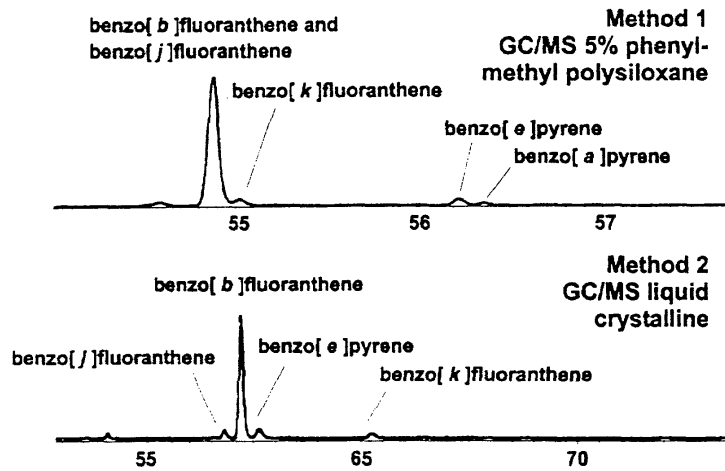
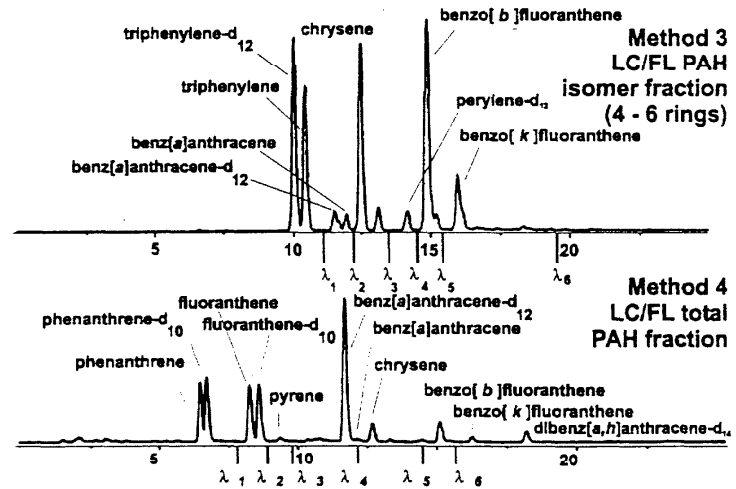


FIGURE 2 GC/MS analyses of SRM 1975 (Diesel Particulate Extract).

FIGURE 3 LC/FL analyses of SRM 1975 (Diesel Particulate Extract). Excitation/emission wavelengths are: top -- $\lambda_0 = 252/352$, $\lambda_1 = 285/385$, $\lambda_2 = 263/358$, $\lambda_3 = 406/440$, $\lambda_4 = 295/450$, $\lambda_5 = 296/405$, $\lambda_6 = 300/500$; bottom -- $\lambda_0 = 249/362$, $\lambda_1 = 285/450$, $\lambda_2 = 333/390$, $\lambda_3 = 285/385$, $\lambda_4 = 263/358$, $\lambda_5 = 295/450$, $\lambda_6 = 295/405$.

in the stationary phase are thought to enable discrimination of isomers on the basis of molecular shape and benzo[*b*]- and benzo[*j*]fluoranthene can be separated (Figure 2). Hence, additional information can be obtained for discrete PAH isomers when this column is used in conjunction with a 5 % phenyl-methyl polysiloxane column.

Similarly, triphenylene can not be determined individually relative to chrysene when a 5 % phenyl-methyl polysiloxane stationary phase is used with GC/MS (Method 1). However, triphenylene can be determined by not only GC/MS analysis with a liquid crystalline column (Method 2), but also by LC/FL analysis of the four-to-six ring PAH fraction (Method 3) (Figure 3), thereby providing additional PAH data for the certification of PAHs in SRMs. Chrysene can also be determined individually by LC/FL analysis of the total PAH fraction (Method 4) (Figure 3).

The results from each of the four analytical methods used for the determination of PAHs in SRM 1975 are summarized in Table I. Results from all four data sets are in good agreement and were combined to provide certified concentrations (expressed as mass fractions) for eight PAHs in SRM 1975 (Table II). Certified concentrations of PAHs in SRM 1975 range from less than 1 µg/g extract to 13.7 µg/g extract for fluoranthene. Certified concentrations are weighted averages where the weighting takes into account the different within-method variabilities. The associated uncertainties are approximate 95 % confidence intervals for these averages. The intervals account for within-method uncertainty, as well as for differences between the results of the analytical methods. A study of homogeneity did not indicate significant variability in concentration among bottles. For all compounds, at least two methods of analysis were used for the determination of their certified concentrations (Table I).

Data from each method of analysis for compounds that overlap with another compound in one of the methods of analysis are in good agreement. The concentration of chrysene and triphenylene in combination determined by Method 1 (GC/MS 5 % phenyl-methyl polysiloxane) agrees with the concentrations of these compounds determined by Method 2 (GC/MS liquid crystalline column) and Method 3 (LC/FL PAH isomer fractions) (Figure 4).

TABLE II Proposed certified concentrations (expressed as mass fractions)* for selected PAHs in SRM 1975 (Diesel Particulate Extract)

PAH	$\mu\text{g/g extract}$
phenanthrene	8.08 ± 0.32
fluoranthene	13.7 ± 0.7
benz[a]anthracene	0.080 ± 0.006
triphenylene	2.37 ± 0.12
chrysene	1.91 ± 0.18
benzo[b]fluoranthene	3.21 ± 0.12
benzo[k]fluoranthene	0.147 ± 0.008
benzo[e]pyrene	0.266 ± 0.024

* See text for description of certified concentrations

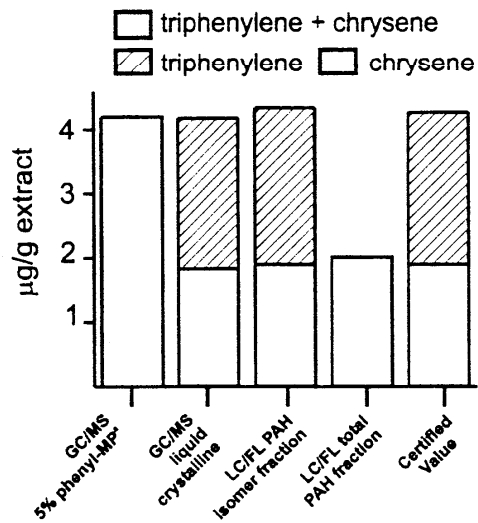


FIGURE 4 Concentrations of triphenylene and chrysene in SRM 1975 (Diesel Particulate Extract)

* MP = methyl polysiloxane.

References

- [1.] S. A. Wise, M. M. Schantz, M. J. Hays, B. J. Koster, K. E. Sharpless, L. C. Sander, B. A. Jr. Benner, and S. B. Schiller, *Polycyclic Aromatic Compounds*, **9**, 209 (1996).
- [2.] S. A. Wise and M. M. Schantz, in *Reference Materials for Environmental Analysis*, edited by R. E. Clement, L. H. Keight, and K. W. Michael Siu (CRC Press, Boca Raton, 1997), Chap. 12, p. 143.
- [3.] D. L. Poster, L. C. Sander, and S. A. Wise, in *The Handbook of Environmental Chemistry, Vol 3 Part I*, edited by A. H. Neilson (Springer-Verlag, Berlin), Chap. 3, p. 77.
- [4.] S. A. Wise, M. M. Schantz, B. A. Jr. Benner, M. J. Hays, and S. B. Schiller, *Anal. Chem.*, **67**, 1171 (1995).
- [5.] M. M. Schantz, R. Dermiralp, R. R. Greenberg, M. J. Hays, R. M. Parris, B. J. Porter, D. L. Poster, L. C. Sander, K. S. Sharpless, S. A. Wise, and S. B. Schiller, *Fresenius J. Anal. Chem.*, **358**, 431 (1997).
- [6.] S. A. Wise, M. M. Schantz, M. J. Hays, B. J. Koster, K. E. Sharpless, L. C. Sander, and S. B. Schiller, *Polycyclic Aromatic Compounds*, **12**, 21 (1997).
- [7.] W. E. May and S. A. Wise, *Anal. Chem.*, **56**, 225 (1984).
- [8.] S. A. Wise, B. A. Benner, Jr., G. D. Byrd, S. N. Chesler, R. E. Rebbert, and M. M. Schantz, *Anal. Chem.*, **60**, 887 (1988).
- [9.] S. A. Wise, L. C. Sander, and W. E. May, *J. Chromatogr.*, **642**, 329 (1993).
- [10.] S. A. Wise, L. C. Sander, H.-C. K. Chang, K. E. Markides, and M. L. Lee, *Chromatographia*, **25**, 473 (1988).
- [11.] L. C. Sander, M. Schneider, C. Woolley, and S. A. Wise, *J. Microcolumn Sep.*, **6**, 115 (1994).